Preconditioning cardioprotection and exercise performance: a radical point of view

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Abstract

It is well known that regular exercise training can reduce the incidence of coronary events and increase survival chances after myocardial infarction. Myocardial beneficial effects are due to the reduction of several cardiovascular disease risk factors, such as high cholesterol, hypertension, metabolic syndrome, obesity, etc. Moreover, exercise can reproduce the so-called “preconditioning”: the capacity of brief periods of ischemia to induce myocardial protection against ischemia/reperfusion injury. Pre- and post-conditioning of the myocardium are two treatment strategies that considerably reduce post-ischemic contractile dysfunction and the amount of necrosis. Paradoxically, reactive oxygen and nitrogen species (ROS and RNS) have been identified as essential cardioprotective signaling molecules, in either pre- or post-conditioning phenomena. Several clues demonstrate that preconditioning may be directly induced by exercise, thus leading to a protective phenotype at cardiac level without the necessity of causing ischemia. Also exercise appears to act as a physiological redox-sensible stress that induces antioxidant beneficial myocardial adaptive responses at cellular level. The purpose of the present work is to review the role played by factors released during exercise in improving exercise performance and in triggering cardioprotection *via* a redox-sensible mechanism.

Keywords: *Exercise performance; Preconditioning; Postconditioning; Endothelial factors; Redox balance.*

Introduction

Cardiovascular diseases (CVDs) will be the major cause of death in the world as a whole by the year 2020, and will determine a human and economic costs that will be unmatched by any other disease [1]. *Physical inactivity* is now recognized as a major risk factor for CVD. For example, the relative risk of coronary artery disease has been estimated to be *circa* 2 fold higher for inactive subjects compared to physically active individuals [2]. Recent esteems suggest that about 12% of the cost of CVD can be attributed to physical inactivity [3], making physical inactivity a multi-billion **€** problem.

Regular exercise is beneficial for the cardiovascular system

A significant amount of time, effort and resources are devoted to methods of prevention trying to reduce the burden that ischemic heart disease poses to our health care system. The notion that *regular exercise* may prevent and cure CVD is supported by numerous epidemiological studies. Physical inactivity is a risk factor for these pathologies and physical activity is cardioprotective. Actually, exercise is one of the oldest therapeutic interventions recommended for the treatment or prevention of diseases. In fact it was recommended by the ancient Chinese, Indians, Greeks, and Romans in various forms [4]. More recently, we can find prescription of regular exercise for the prevention of CVD already in the 1850s in Scotland, Scandinavia and Germany [4], as well as in 1904 in the USA [5]. Nowadays the concept that regular exercise confers protection against coronary disease can be traced to the seminal work of Morris and co-workers [6] and it has been extensively investigated since in a number of studies which have demonstrated that regular exercise is beneficial for the cardiovascular apparatus. Regular physical activity (for about 2-4 years),

was associated with a 27% reduction in total mortality and a 31% reduction in cardiac mortality [7]. Moreover, exercise has been suggested as a useful tool in rehabilitation for stable coronary insufficiency and after infarction [8-10], and there are evidences supporting the beneficial effect of regular physical activity in patients with coronary disease [11]. It has been reported that exercise reduces the incidence of arrhythmias [12-14], improves coronary vascular reactivity [15-17] and decrease myocardial stunning [18,19] in hearts experimentally exposed to ischemia/reperfusion (I/R). In humans, exercise decreases the incidence of myocardial infarction and increases the chances of survival after coronary events [20-30]. Furthermore, it is well established that *exercise capacity* is a good predictor of reduced risk of death from any cause in both healthy subjects and in those with CVD [31].

However, the mechanisms through which regular exercise protects against chronic and acute CVD have not yet been completely elucidated.

The putative mechanisms of cardiovascular protection by exercise

It has been proposed that physical training operates by:

1) *improvement of endothelial function*. Indeed, the vasculature is the largest organ in the body and endothelium is important in regulating some key functions in homeostasis such as platelet aggregation, immune responses, and vascular permeability. Moreover, the endothelial cells produce numerous substances, including nitric oxide (NO), which is important in regulating vasomotor function, thereby determining blood flow distribution to each tissue and in maintaining the health of the vascular wall [32]. It is now established that exercise improving endothelial functions and endothelial-dependent vasodilatation increases gene expression for endothelial NO synthase (eNOS) [16,33-36].

2) *reduction of vascular resistance* and *structural adaptations in the coronary tree* (*i.e.* increased number of capillaries and number and size of arteries and arterioles), thereby enhancing the blood transport capacity at this level [15-17];

3) *reduction of several risk factors* related to cardiovascular pathologies, including high blood pressure, dyslipidemia, obesity, insulin resistance, and autonomic deregulation [3,8,37,38].

4) cardioprotection by exercise includes also *modified gene expression*. In fact, it has been demonstrated that even mild exercise can trigger gene modifications that may become relevant for cardioprotection [39,40]. In particular, exercise induces the increase of cytoprotective molecules, including heat shock proteins (HSPs) and antioxidant defense (see below) [19]. However, there is a need for studies that utilize an integrated "omics" approach (genomic, proteomic and metabolic analysis) with subsequent robust network bioinformatics analysis to identify key regulatory networks, signaling hubs and confounders in response to exercise as well as to other conditioning stimuli.

Similarity between preconditioning and exercise

Besides exercise, a growing number of strategies have been described as possible treatment to protect the heart from I/R injury. Among them, one the most powerful is the so called "*ischemic preconditioning*" phenomenon, first described by Murry et al. [41], where a series of short periods of ischemia separated by brief episodes of reperfusion before a long *index/infarcting* ischemia greatly reduced infarct size. Since then, several researchers have put forward the idea that cardiovascular protection by exercise and by ischemic preconditioning may share several mechanisms.

The similarity and differences between exercise and preconditioning have been analyzed in some recent reviews [42-48]. Similarly to exercise, conditioning protocols may affect gene expression, as elegantly reviewed by Ferdinandy's group [49]. Here we consider only some aspects of the mechanisms of preconditioning and exercise.

After a general introduction on preconditioning and exercise we will specifically analyze mitochondrial and endothelial function as they are of paramount importance both in exercise and preconditioning. In particular, we will describe *a)* the role of some autocrine and paracrine factors in influencing endothelial function in exercise and preconditioning, *b)* the role of mitochondrial reactive oxygen species (ROS) in these two phenomena, *c)* redox aspect and interaction with gasotransmitters on the endothelial function during exercise and preconditioning.

Conditioning by brief ischemia as protective procedure

Ischemic Preconditioning

As said, one of the most promising approaches in reducing myocardial I/R injury is ischemic preconditioning (I-PreC), a phenomenon induced by repetitive brief episodes of ischemia during early reperfusion before a prolonged ischemic insult [42,50-52] In recent years, it has been shown both in the experimental and clinical settings that the infarct-limiting effect of ischemic preconditioning is due to the autocrine/paracrine effects of ligands released by the preconditioned tissue (see below). It is likely that ligand formation and release is favored by oxygen tension drop in the tissue, which occurs also in exercise [53]. Of note, the hypoxia also promotes an increase of mRNA levels of protective expression of

hypoxia-inducible factor 1 (HIF-1) target genes, such as HSPs (for extensive reviews, see [49,54]). However, it has been suggested that hypoxic preconditioning is due to the reoxygenation rather than to hypoxia *per se* [55].

The Trigger phase: the ligands may couple to G-protein-linked receptors, or tyrosine kinase receptors, or may activate directly intracellular signaling pathways, including kinases, such as protein kinase C (PKC), nitric oxide synthase (NOS), mitochondrial ATP-sensitive potassium channels (mKATP), which may promote ROS production. Nitric oxide and ROS may react to obtain reactive nitrogen species (RNS). Both ROS and RNS have a fundamental signaling role. In fact, antioxidant given in this phase avoids the induction of preconditioning cardioprotection [42,43,50-52,56].

The mediation phase: cardioprotective mechanisms and pathways similar to those observed in the trigger phase are operative in the reperfusion phase. These ROS/RNS signaling, as well as protective signaling pathways, namely cGMP/PKG (cyclic guanosine monophosphate/Protein kinase G), RISK (Reperfusion Injury Salvage Kinase) and SAFE (Survivor Activating Factor Enhancement), which converge on mitochondria. Thus, for the endogenous ligands the final targets of the protective pathways are the mitochondria, where the signaling induces protection by preventing mitochondrial permeability transition pore (mPTP) formation, which is considered the end-effector of the protective pathway. Thus, regardless the fact that the ligands are formed during the pre-conditioning procedure, the real protection occurs against reperfusion injury, which follows the index ischemia [43,56].

In brief, ***three mainstream cardioprotective pathways*** are described, namely the *cGMP/PKG pathway*, which starts from nitric oxide formation and guanylyl cyclase (GC)

activation, the *RISK pathway*, which includes activation of protein kinase B (PKB, also known as Akt) and extracellular signal-regulated kinase (ERK)1/2, and the *SAFE pathway*, which requires the activation of the signal transducer and activator of transcription 3 (STAT3). These protective pathways, here only mentioned, have been described in several excellent reviews, to which the reader is kindly redirected; see for example [43,52,57-61].

Other conditioning protocols

Besides ischemic preconditioning the heart may be "conditioned" by other approaches. *Ischemic postconditioning* can be triggered by short cycles (a few seconds) of I/R at beginning of reperfusion, immediately after ischemia. It is a protective mechanisms observed in early phase of reperfusion after an infarcting ischemia and has also shown to be protective against I/R injury [59,60,62-64]. Similarly to preconditioning, postconditioning reduces: infarct size, apoptosis, post-ischemic arrhythmias and endothelial-dysfunction/activation.

Cardioprotection by *pharmacological postconditioning* has also been described. It is obtained with the infusion of some of the agents able to induce preconditioning, but these agents must be applied very soon in reperfusion, because the first minutes of reperfusion are critically important [59,60,65,66].

More recently, *remote ischemic pre-, per- and post-conditioning* have also proved to be protective against cardiac I/R damage [67-73]. These approaches consist in subjecting a limb or organ, remote from the heart, to brief cycles (a few minutes) of ischemia/reperfusion, *prior to* or *during* or *after* cardiac ischemia, respectively (Fig. 1).

Cardiac ischemic and remote preconditioning have been shown to induce both early (the above described phenomenon) and delayed cardioprotection [73-76]. This delayed protection, is also named *"second window of protection"* (SWOP).

While the early protective phenomenon is active immediately after conditioning ischemia and lasts for about 2-3 hours, SWOP begins 12-24 hours after the initial conditioning ischemia and lasts 72-90 hours conferring a delayed cardioprotection [77,78]. This late phase of protection is particularly interesting in a clinical perspective because of its sustained duration. Besides duration, other differences between early preconditioning and SWOP exist. For instance, SWOP is more effective than early preconditioning in attenuating myocardial stunning, whereas the early phase is characterized by a pronounced infarct-sparing effect [42,79,80]. Notably, a cardioprotective role of HIF-1 and related target genes have been demonstrated in both delayed and early phase of preconditioning as well as in remote limb ischemic preconditioning [43,81].

The redox signaling in cardioprotection (preconditioning and postconditioning)

Here we report some details about the role of ROS/RNS signaling in pre- and postconditioning of the heart. Transient pre-ischemic ROS/RNS formation is of paramount importance in triggering preconditioning cardioprotection. Whether redox signals arise during the reperfusion phase which follows the brief preconditioning ischemia [82] or during preconditioning hypoxia/ischemia itself [83], is a matter of controversy. Although preconditioning modulates ROS/RNS production during the infarcting ischemia [82-85], it also limits, but not avoids, ROS/RNS production in reperfusion [52,58,61,83,85,86].

Actually, the acidosis and redox signaling at the beginning of reperfusion contribute to trigger cardioprotective pathways, comprising several processes of activation/inhibition of key enzymes. Moderate acidosis plays a critical role in the prevention of mPTP opening. In fact, similarly to ischemic postconditioning a slight acidosis treatment in the initial phase of reperfusion is protective. Acidosis cardioprotective role has also been clearly demonstrated in postconditioning experiments and has been attributed to the direct action on mitochondria, where mPTP formation is prevented [87]. Consistently, transient preconditioning with acidosis is cardioprotective [88]. Preconditioning not only decreases tissue acidosis and anaerobic glycolysis during the subsequent sustained ischemic period [84], but also avoids quick pH recovery in the early reperfusion [58]. Therefore, in the initial phase of reperfusion a slight acidosis plays a critical role in the cardioprotection against reperfusion injury both in pre- and post-conditioning. Thus preventing mPTP opening, slight acidosis limits ROS production and avoids the ROS-induced ROS release (RIRR) phenomenon, which is irreversible and will lead inevitably to cell death. Therefore, ROS/RNS signaling is also cardioprotective at the beginning of reperfusion. In fact, ROS/RNS are double-edged swords whose harmful role is well known in I/R context. Nevertheless their cardioprotective role has been unequivocally demonstrated in postconditioning experiments. This redox sensible protection has been attributed to the direct and indirect modulation of pro-survival kinases activity, including PKC [52,61,82,89] (Fig 2). In summary, both in pre- and post-conditioning the persistence of a slight acidosis and a slight production of ROS/RNS in the early phase of reperfusion play crucial roles in the cardioprotection against I/R injury.

The redox signaling in exercise

It is clear that acute exercise modifies redox homeostasis in every fluid, blood cell, tissue and organ. In fact, several studies have found alterations in redox homeostasis after acute exercise in several tissues [90-100] including the heart [101].

There are several types of exercise and ROS/RNS production may be typical for each type of exercise protocol and this makes this issue particularly complex. From the point of view of redox signaling, it may be important/useful to divide the acute exercise in non-muscle- and muscle-damaging exercise. It seems clear that *non-muscle-damaging exercise* induces changes in redox homeostasis that lasts in tissue and blood a few hours after the exercise bout [102-106]. This form of exercise seems more similar to the ischemic conditioning, where brief non-damaging ischemia triggers protection. Exercise may be a paradigmatic example of the beneficial effects induced by transient oxidative signaling [107-109], which also comprises an up-regulation of eNOS [110,111] and other cytoprotective molecules, including HSPs. HSPs up-regulation occurs as a consequence of both acute exercise and, especially, of chronic cardioprotective exercise, and persists over training cessation [112,113].

Here a first question arises: *is exercise-induced cardioprotection due to an enhanced ability to produce ROS/RNS or to an ameliorated capacity to scavenge ROS/RNS?*

It must also be considered that amount of products deriving from the anaerobic metabolism (such as ADP, adenosine, lactate *etc.*) is released into the blood during exercise, even without the need of flow reduction used to cause remote preconditioning [114-116]. Among these substances in the blood are also found factors modifying the redox homeostasis [102-106].

Thus a second question arises: *are these substances and factors produced during exercise by remote organ to trigger cardiac preconditioning?*

To the best of our knowledge there is not a definitive answer to these two questions. Nevertheless, for what concern the second question, it has been recently demonstrated that dialysate plasma from humans undergoing high-intensity exercise reduced infarct size in isolated rabbit hearts after ischemia-reperfusion injury. This phenomenon was also present with plasma from humans exposed to remote ischemic preconditioning [117]. These data suggest that exercise-induced cardioprotection is, at least in part, similar to remote conditioning, *i.e.* it is mediated by systemic release of one or more humoral factors reaching the heart. Similar results were also obtained in the mice heart perfused with dialysate plasma from highly trained humans (swimmers) undergoing a protocol of ischemia-reperfusion to trigger the remote preconditioning phenomenon. In this study, along with the infarct reduction effect in the mice heart, the remote ischemic preconditioning maneuvers were also able to enhance the athletic performance during swimming [118].

All together the above data support an important role for the substances produced by "remote organs" in protecting the hearts also during exercise. However the metabolic demand of the heart increases almost in parallel with exercise effort, thus a contribution from the autocrine/paracrine effects of factors released by the heart itself is more than likely.

For what concern the first question (*is exercise-induced cardioprotection due to an enhanced ability to produce ROS/RNS or to an ameliorated capacity to scavenge ROS/RNS?*). Either phenomena may be true. The predominance of one or the other mechanism may depend from several factors.

As candidates for triggering cardioprotection after acute exercise, the activation of adenosine, bradykinin and/or opioid receptors, and/or surges in inflammatory adipocytokines, as well as transient ROS/RNS production have been put forward (Fig. 2).

Several studies reported that the improvements in cardiac function and infarct-sparing effects triggered by exercise, are lost when antioxidants are assumed by the individuals during the exercise bouts [119-121]. Moreover, exercise increases the activity of myocardial NADPH oxidase (a ROS producers), and its inhibition abrogated the cardioprotective effects of acute exercise [122]. A small ROS burst may increase antioxidant buffering capacity by promoting gene expression and protein synthesis, similar to what observed in skeletal muscle [123].

In the heart there are several antioxidant enzymes, which can upregulate their activity after exercise. However, it seems that this is not a feature of acute exercise, which may be, however, able to induce preconditioning-like cardioprotection. In fact in animals voluntary free wheel running [124] and low-intensity treadmill running [125-127] do not increase myocardial *manganese-dependent superoxide dismutase* (MnSOD) levels. Moreover, neither MnSOD mRNA [128] nor protein levels [129] are augmented after acute exercise. A clear upregulation of MnSOD, in both activity and enzyme expression, can be seen only in studies examining exercise protocols of longer duration [125,130-133] a feature that appears to be preserved in the aged heart [132,133]. While, heightened enzymatic scavenging of superoxide anion (O_2^-) seems a feature of adaptation to chronic exercise, an enhanced enzymatic set of hydrogen peroxide (H_2O_2) scavengers does not seem to be necessary for exercise-induced cardioprotection. In fact, only a few studies have reported and enhanced *catalase* activity in the heart [130], with most studies reporting no difference

in cardiac catalase and *glutathione peroxidase* activities after exercise training [14,124,134-136]. Moreover, myocardial *thioredoxin* appears to be unaffected by exercise [134]. Finally, whether *glutathione reductase* is involved in exercise-induced cardioprotection remains to be clarified. Some studies observed enzyme increase with exercise [132,137] whereas others reported no change [124,131,134].

Moreover, the results with exogenous antioxidants are controversial. In fact, while several studies suggest that antioxidant supplementation induces a positive effect [138,139], others studies report either a negative [140,141] or a neutral effect [142-144] on exercise performance. Similarly, several studies have reported that antioxidant supplementation limits oxidative stress [145,146], others report a pro-oxidant effect [147] and others describe a neutral effect on redox homeostasis [144].

In summary, it seems that a modest increase in reactive species may trigger cardioprotection, whereas this positive effect may be reversed at higher ROS/RNS concentrations in a dose-dependent manner. This is in line with the idea that antioxidant supplementation on redox homeostasis are dependent on the antioxidant concentration and effect: too much generation of ROS/RNS may be harmful whereas modest generation may be beneficial [148].

The beneficial effects of exercise on properties of endothelial cells and vasculature: a redox perspective.

Endothelium plays a role of paramount importance in exercise regulation [149,150]. Endothelial cells (ECs) form a multifunctional *signal-transducing surface*, which regulates several fundamental processes, including blood flow and pressure response to exercise. ECs

present numerous ion channels and enzymes and produce many factors affecting the response to exercise. Some of these factors are produced “on demand” (NO, endothelins, PGI2, and apelin), whereas others compounds (tissue plasminogen activator, von Willebrand factor, tissue factor pathway inhibitor, etc.) are stored in granules and released by exocytose, tonically and in response to various stimuli. Among stimuli there are both chemicals/neurotransmitters (such as apelin, histamine, acetylcholine, angiotensin, bradykinin, ATP, ADP, thrombin, growth factors) and mechanical stimuli (such as increased pulsatility and shear stress) [149-152]

The increase of shear stress and pulsatile pressure during acute exercise represent the mechanical stimulation of the arteries and arterioles. These two important mechanical stimuli “augment” the endothelial regulation of vascular tone to ameliorate blood flow where needed. Shear stress and cyclic tension may thus operate to maintain the viability and phenotype of normal endothelium [153,154]. During acute exercise, in particular, the mechanical forces acting on ECs induce the release of NO and other vasodilator factors, that in some vascular district (see infra) dilate preferentially arteries and more proximal arterioles and may act as a local vasodilator amplifier of the metabolites released by the muscle, which act mainly on smaller arterioles [155,156]. Therefore metabolites (such as adenosine or K^+ acting on K^+ ATP sensible channels) and endothelial factors (*e.g.* NO acting also *via* cGMP) present a synergistic action in determining in specific vascular regions their vasodilator effects.

Even when the function of the endothelium is compromised, as it can occur in aging and/or exposure to risk factors for cardiovascular disease, exercise represents an important and beneficial effect and this effect is correlated with improved production of NO [157]. The

beneficial effects of exercise, such as increased vasodilators and reduced vasoconstrictors, and lowered blood pressure, may contribute to reverse the endothelial dysfunction possibly through an increased availability of NO due to an increased production and/or inhibition of NO degradation and scavenging by lowered lipoprotein levels. Importantly, the improvement of endothelial function during exercise is correlated with an increased extracellular superoxidismutase (ecSOD) activity leading to a reduction of ROS and increasing NO half-life [110,158,159]. In fact ROS have a major role in NO degradation. It is likely that exercise inducing the formation of NO and scavengers of O_2^-, limits the formation of the deleterious peroxynitrite ($ONOO^-$) and favors positive processes of S-nitrosylation [52,56]. However, as said above, it is not clear whether and how different exercise types and training alters antioxidant defense system and NO availability in humans. Intriguingly, animal studies suggest that at certain point of training an increased NO availability is no longer detectable [160,161]. It has been suggested that short-term exercise training enhances NO production and bioactivity to induce vasodilation and to buffer increased shear stress. After extended training increased NO production, and possibly other factors, induces structural changes to the vessel resulting in an increase in lumen diameter. Hence, shear stress is constantly normalized and endothelial NO production returns towards initial levels [161]. However, a regional differences in effects must be taken in account as endothelial and/or vascular adaptations are specific to certain regions, regardless if the skeletal muscles of the considered region are active or not during training bouts [157,162,163].

Autocrine/paracrine protective effects of factors released during I-PreC or Exercise

Several substances released by exercising tissue may be responsible of the observed protective effects. Among these are included adenosine, apelin, bradykinin, opioids and gasotransmitters (CO, H_2S and NO); several of these have been widely described in several recent reviews [42-48,52,57-61,164], here we consider only apelin and H_2S giving particular emphasis to their redox signaling role.

Apelin

Adipokines or adipocytokines are active polypeptides produced by the adipocytes. They exert a plethora of functions in physiological and pathological conditions and several of them are considered as predictor markers for cardiovascular diseases [165].

Production and concentration of various adipocytokines, such as apelin, visfatin, resistin. leptin, adiponectin, IL-6, MCP1 and TNF-α are influenced by physical exercise. Because of the role of exercise in protecting myocardium against I/R injury, it may be argued that changes in the production of one or more adipocytokines could affect the exercise-induced protection. Actually, the exercise-induced changes in leptin, TNF-a, adiponectin, IL-6 and MCP1 release have already been extensively discussed in recent reviews [166,167].

Here we focus our attention on apelin, an adipokine widely studied in cardiovascular system for its cardiac protective effect. The most active isoforms on cardiovascular system are apelin 13 and, to a lesser extent apelin 36, while the predominant in the heart and plasma is the pyroglutamyl apelin 13 ((pyr)-apelin 13) [168-170]. Adipose tissue is not the only source of apelin. It is also expressed in various organs and tissue, as, e.g., heart (right

atrium), lungs, kidney, liver, gastrointestinal tract, brain, adrenal gland and endothelium of large conduit vessels and endocardium [171,172].

On the cardiovascular system, apelin acting on its own receptor (*i.e.* APJ, a G protein coupled receptor) induces vasodilatation and cardiac inotropy, attenuates arrhythmias and I/R injuries, and promotes angiogenesis. The inotropic effect of apelin has been considered a component of the response to increases in pre- and afterload [172]. Nevertheless, apelin inotropic efficiency is a matter of controversy because apelin was seen to produce either a powerful [168,173,174] or a slight (18%) and brief (1-3 min) increase in left ventricular developed pressure [170,175-177].

As above-mentioned and similarly to other endothelial factors, apelin may be released by endocardial endothelium and vascular endothelium after an increase of shear stress [172]. In fact, it has been reported that an exercise-induced increase in shear stress triggers apelin-mediated vasodilatation and improve cardiac contractility [172]. Actually, the apelin vasodilator effect is endothelium- and NO-dependent [168,177,178]. In fact, in the presence of endothelial dysfunction or NOS inhibition, apelin acts directly on smooth muscle causing vasocostriction *via* a PKC activation and an increase in intracellular calcium concentration [177,179].

Intriguingly, it has been observed that aerobic exercise induces an increase of NOS mRNA expression and NO production, together to an increase in plasma apelin concentration [180]. This is in line with the observation that the *i.v.* administration of apelin induces NO production and promotes eNOS mRNA expression in vascular endothelial cells [181], *via* PI3K/Akt pathway [182].

Apelin plasma level doubled after training with repeated aerobic exercise in both middle-age and old healthy subject [180]. Moreover, in spontaneous hypertensive rats, which were characterized by a down-regulation of apelin, swimming training induced an increase of apelin and APJ levels in plasma, myocardium and aorta, together with a reduction in blood pressure. These results suggest that the effect of training on hypertension may be mediated by an up-regulation of apelin/APJ system [183].

An increase of apelin concentration was observed in plasma of type 2 diabetes patients, after training with aerobic, but not with resistance exercise. In these patients, aerobic training induced also an increase in insulin sensitivity and attenuated carotid intima-media thickness progression [184]. It this study there was no difference in apelin level between healthy and diabetic subjects before training. On the contrary Krist *et al.,* [185] report a higher apelin mRNA expression in adipose tissue and concentration in plasma of diabetic patients. Since in the studied diabetic cohort the fat mass percentage was significantly higher than in the control, it can be argued that the increased apelin concentration was related to the overweight.

Consistent with what described right above, apelin plasma level is augmented in obese subjects, but decreased in women when aerobic exercise training causes a reduction of body fat mass [186]. However, this does not occur in males in spite of a reduction in fat mass; interestingly, apelin mRNA expression does not change in adipose tissue but increased in skeletal muscle [187].

In the case of exercise training also a protection against I/R injury takes place. The protection consisted in the limitation of the infarct size and the improvement of post-ischemic mechanical recovery [188]. Due to the timing of exercise training, this kind of

protection must be taken as a sort of preconditioning. However, in isolated rat hearts, protection was obtained only if apelin was infused at beginning of reperfusion (that is postconditioning), but not if given before the infarcting I/R [177]. Since exogenous apelin acts as postconditioning agent only, it may be argued that the relevant endogenous apelin in trained subjects is acting in reperfusion.

Apelin exerts its protective effect against I/R by delaying the opening of mPTP via PI3K/Akt and NOS signaling pathway [189]. Apelin displays also antioxidant properties. In fact, ROS production was reduced in cardiomyocytes when apelin was added to the medium before hypoxia-reoxygenation [190]. Attenuation in O_2^- and $ONOO^-$ levels was seen also in isolated hearts when apelin was given before I/R [191]. Moreover, apelin affects O_2^- and H_2O_2 production in mitochondria [192]. These antioxidant effects of apelin seem to be due to the recovery of the activity of antioxidant enzymes, such as Cu- and Zn-SOD, catalase, and glutathione peroxidase, in myocardium after I/R [191,193]. In particular, the involvement of catalase in mediating apelin-induced removal of H_2O_2 has been observed also in pressure overload mice hearts, where this effect was also seen to prevent myocardial hypertrophy [194]. As reported above, this is in part at variance with the antioxidant effects of chronic exercise, in which SOD expression increases, but peroxide scavenging activities are not usually up-regulated. Notably, apelin effects on redox conditions are also mediated by pro-survival signaling pathways, comprising PI3K, PKC, and/or mKATP channels [191] (Fig 2). Nevertheless, it has been reported that after 2 weeks of apelin injection in male rats, an increase in mitochondrial enzyme activity and respiratory chain protein content can be observed in triceps, but not in soleus muscle and in myocardium [195]. The absence of this

increase was attributed to the already high levels of mitochondria in high oxidative tissue, such as the myocardium.

Gasotransmitters, physical exercise and cardioprotection

The role of gaseous signaling molecules in physiology and pathophysiology has been extensively described in recent literature [*e.g.* 33-35,162-164,196]. Concomitantly, gasotransmitters (CO, NO and H_2S) effect on physical exercise has received scientist attention with particular focus on NO. By definition, gasotransmitters are toxic at high concentrations whilst indispensable at low doses that are constitutively produced [164,196]. Impaired basal production of these molecules leads to altered cardiovascular functions and all gasotransmitters have been involved in cardioprotection [164]. In addition, as said, there are a plethora of evidences for cardioprotection conferred by physical exercise *via* NO metabolites [164,197] suggesting an important role of gasotransmitters in exercise-induced cardiac protection. Although NO is a paramagnetic species and as such can act as gasotransmitter in short distance (in neighboring cells) during organ's conditioning procedures, its halftime in the circulation is brief and would not allow long traveling distance as remote conditioning would need. Nevertheless, nitrite may represent a major bioavailable pool of NO, on which hemoglobin may act as a nitrite reductase, thus potentially contributing to NO-mediated hypoxic and exercise vasodilatation, even in remote organs [198]. Despite of the large amount of research about CO and its cardiovascular effects [164], very few studies considered a possible involvement in the cardioprotective effect of physical exercise. The advent of CO-releasing molecules (CO-RMs), as compounds capable of carrying and liberating controlled quantities of CO, may help to overcome the dangerous limitations of CO as gasotransmitter [199]. In particular,

Vanergriff *et al* provided the first evidence for CO-MP4, a CO-carrier agent, as deliverer of CO to the circulation is able to reduce I/R injury in rats [200]. Intriguingly, an increase in the mRNA levels of heme-oxygenase-1 (HSP32), the endogenous CO generator with antioxidant properties, has been observed in skeletal muscle after repetitive contractions [201]. This may underlie an inducible antioxidant pathway in muscle responsive to metabolic stresses associated with repeated muscle contractions. However, to the best of our knowledge, an upregulation of heme oxygenase-1 (HSP32) after exercise in cardiac muscle has not been described so far.

NO and Physical exercise. As seen above, NO is the member of the gasotransmitters family which has received the major attention. Its beneficial effects in terms of endothelial function, response to ischemia and blood pressure regulation are also involved in the adaptation to physical exercise. A pivotal mechanism against myocardial I/R injury, which deserves few words of discussion, relies on the ß-adrenergic stimulation of NOS. In fact, circulating catecholamines are elevated upon voluntary physical exercise and eNOS activity increases *via* stimulation of ß3-adrenergic receptors and after injection of epinephrine [202]. However, while ß1 and ß2 stimulation increases inotropism and lusitropism, ß3 receptors are shown to enhance production of NO from eNOS leading to a negative inotropic effect [203]. However, another putative mechanism is based on the sympathetic drive that, during physical exercise, stimulates ß2 receptors both on endothelial and cardiac cells upregulating eNOS activity. In fact, recent evidences show how ß2 stimulation can activate eNOS *via* a pro-survival Src kinase-PI3K/Akt-dependent pathway although independently from cAMP/PKA, MAPK, and AMPK [204]. As said above NO may exert both positive and

negative inotropic regulations [164], thus an interesting relation exists with sympathetic signals, which deserves further attention.

***H_2S and Physical exercise*.** Hydrogen sulfide has been shown to influence exercise capacity during sub-maximal and maximal exercise [205] and, concomitantly, several evidences confirm a potent cardioprotective effect against ischemia\reperfusion injury and a mediatory role in both pre- and postconditioning [164,206,207]. A possible link between the two effects probably relies in the ability of H_2S to influence mitochondrial activity. At sub toxic doses, H_2S can serve to the respiratory chain through Sulphide Quinone Reductase [208]. Considering these experimental evidences it seems reasonable to assume a potential role for H_2S in physical exercise-mediated cardioprotection. However, probably due to the recently emerged attention to the latest member of the gasotransmitters family, there is no animal study showing a link between the subjects. The only report that somehow approaches the problem is Tiagy's review on the effect of H_2S on myocytes metabolism [209]. Unfortunately, aside from analyzing power output capacity upon inhalation of different concentration of H_2S, there is no evidence linking H_2S mediated cardioprotection and physical exercise.

Along with the protective action on the myocardium, emerging evidence supports the notion that preconditioning may be beneficial also for the skeletal muscle, thereby improving exercise performance.

Preconditioning and its potential effects on exercise performance

An increasing number of studies have been published and have demonstrated that I-PreC can effectively enhance performance in several kinds of exercise [118,210-212]. However,

these beneficial effects have not been unanimously proved, and some studies reported uncertain [213-216] or even negative effects [217]. These different outcomes could be the consequence of differences in the maneuvers to induce preconditioning, in the different exercise duration and modes, and in timing between preconditioning and exertion. We cannot rule out that the differences are due to the redox status of the tissue.

De Groot and co-workers [210] were among the first to test the I-PreC effect on exercise performance. They found that skeletal muscle I-PreC increased maximal oxygen uptake and power output during maximal cycling performance in healthy and physically fit subjects. The increment in subjects' performance after I-PreC was slight but significant. Similarly, in another investigation it has been demonstrated that skeletal muscle I-PreC slightly improved maximal performance in highly trained swimmers [119]. Of note, these authors were able to demonstrate that the dialysate plasma from the preconditioned athletes could reduce the infarct area of perfused mice hearts. The possibility to enhance exercise performance by I-PreC was substantially confirmed by Crisafulli and co-workers [211] during maximal cycling. However, these investigators failed to detect any benefit of I-PreC during all-out testing, a kind of exercise mostly related to athletes' anaerobic capacity. A very recent paper has confirmed the fact that I-PreC can not ameliorate athletic performance during anaerobic testing [218]. Another very recent investigation confirmed the positive effect of I-PreC on exercise performance and fatigue perception [219]. Collectively, reported data seem to suggest that I-PreC can only produce little improvements in exercise performance (in the order of 2-3%) and that this effect is more pronounced in aerobic exercise (running, swimming, cycling etc.) than in power and sprint performance. It should however be borne in mind that athletic competitions are often decide by a small margin of

differences. Therefore, from an athletic performance point of view improvements of 2-3% may be substantial. That is, increments of this order is highly relevant in elite athletes' competitions.

As far as the mechanisms by which I-PreC operates to increase exercise performance, to date none has provided any definite explanation. It has been suggested that I-PreC produces a more efficient muscle contraction, thereby promoting an ATP-sparing effect which, in turn, led to a larger work load per oxygen consumed. This effect has in fact been demonstrated in a pig model of I-PreC [220]. Authors speculated that the ATP-sparing effect afforded by I-PreC may occur because of a tightening of excitation-contraction coupling and a reduction in futile ion pumping. It is also possible that I-PreC reduces the feeling of fatigue, thus allowing the subjects to exercise longer [211]. This fact could be related to the desensitization of groups III and IV nerve endings in the muscle, which act as mechanic and metabolic sensors. The reduction of signals by these nerve endings may decrease the central sensation of fatigue and could at least in part explain the increased performance. Alternatively, I-PreC-related enhancement in exercise performance may be the consequence of improvements in peripheral vascular functions. Indeed, I-PreC can increase NO production, which induces vasodilation, increments in muscle blood flow and oxygen delivery, thereby improving muscle aerobic capacity [48,74]. This latter mechanism may be particularly relevant in the adaptive effects of physical training, where an enhanced exercise-induced NO production is usually observed in some vascular districts [15,33,35].

Finally, similar to exercise, I-PreC can induce an increase in ROS/RNS production [91,92,96.], followed by an antioxidant and survival proteins up-regulation in both training

and late preconditioning [78,79,133,136], thus reinforcing the concept that I-PreC and exercise can act in the same biochemical pathways. However, we should keep in mind that ROS/RNS have multi-phasic effects on the contractile function of skeletal muscle. The low ROS levels present under basal conditions are essential for normal force production. Selective depletion of ROS from unfatigued muscle by use of SOD or catalase causes force to fall. Conversely, modest ROS supplementation causes force to increase. This positive effect is reversed at higher ROS concentrations; force production falls in a time- and dose-dependent manner [221]

In conclusion, preconditioning has similarity with exercise and both induce a great benefit against I/R injury *via* several mechanisms, which comprises a redox modulation. However, I-PreC induces a small benefit on exercise performance, mainly during aerobic efforts. Further investigation is warranted to better clarify effects and mechanisms of both phenomena: exercise and preconditioning.

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References

1. Murray CJ, Lopez AD (1997). Alternative protections of mortality and disability by cause 1990-2020: global burden of disease study. Lancet 349:1498-1504.
2. Roberts CK, Barnard RJ (2005). Effects of exercise and diet on chronic disease. J Appl Physiol 98:3-30.
3. Yusuf S, Hawken S, Ounpuu S, et al (2004). Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the Interheart Study): case-control study. Lancet 364:937–952.
4. Light S. History of therapeutic exercise. In: Therapeutic Exercise, edited by Light S. New Haven, CT: Elizabeth Licht, 1958, p. 380–422
5. Anders J. The Schott method of treating diseases of the heart and blood vessels. In: Journal of Advanced Therapeutics, edited by Snow W. Boston, MA: Chatterton, 1904, p. 455–460
6. Morris JN, Heady JA, Raffle P, et al (1953). Coronary heart disease and physical activity of work. Lancet 265:1053-1057
7. Kumar A, Kar S, Fay WP (2011). Thrombosis, physical activity, and acute coronary syndromes. J Appl Physiol 111:599-605.
8. Shephard RJ, Balady GJ (1999). Exercise as cardiovascular therapy. Circulation 99:963-972.
9. Leon AS (2000). Exercise following myocardial infarction. Sports Med 29:301-311.

10. Hambrecht R, Walther C, Möbius-Winkler S et al (2004). Percutaneous coronary angioplasty compared with exercise training in patients with stable coronary artery disease. Circulation 109:1371-1378.
11. Pedersen BK, Saltin B (2006). Evidence for prescribing exercise as therapy in chronic disease. Scand J Med Sci Sports 16:3-63
12. Noakes TD, Higginson L, Opie LH (1983). Physical training increases ventricular fibrillation thresholds of isolated rat hearts during normoxia, hypoxia and regional ischemia. Circulation 67:24–30.
13. Hamilton KL, Quindry JC, French JP, et al (2004). MnSOD antisense treatment and exercise-induced protection against arrhythmias. Free Radic Biol Med 37:1360–1368.
14. Quindry JC, Schreiber L, Hosick P, et al (2010). Mitochondrial KATP channel inhibition blunts arrhythmia protection in ischemic exercised hearts. Am J Physiol Heart Circ Physiol 299:H175–H183.
15. Laughlin MH, McAllister RM (1992). Exercise training-induced coronary vascular adaptation. J Appl Physiol 73:2209-2225.
16. Laughlin MH, Pollock JS, Amann JF, et al (2001). Training induces nonuniform increases in eNOS content along the coronary arterial tree. J Appl Physiol 90:501–510.
17. Brown DA, Jew KN, Sparagna GC, et al (2003). Exercise training preserves coronary flow and reduces infarct size following ischemia-reperfusion in rat heart. J Appl Physiol 95:2510–2518.

18. Bowles DK, Farrar RP, Starnes JW (1992). Exercise training improves cardiac function after ischemia in the isolated, working rat heart. Am J Physiol Heart Circ Physiol 263:H804–H809.
19. Locke M, Tanguay RM, Klabunde RE, et al (1995). Enhanced postischemic myocardial recovery following exercise induction of HSP 72; Am J Physiol Heart Circ Physiol 269:H320–H325
20. Froelicher V, Battler A, McKirnam MD (1980). Physical activity and coronary heart disease. Cardiology 65:153-190.
21. Morris JN, Everitt MG, Pollard R, et al (1980). Vigorous exercise in leisure-time: protection against coronary heart disease. Lancet 2:1207-1210.
22. Eichner ER (1983). Exercise and heart disease. Am J Med 75:1008-1023.
23. Paffenbarger RS, Hyde RT, Wing A, et al (1986). Physical activity, all-cause mortality, and longevity of college alumni. N Eng J Med 314:605-613.
24. Powell KE, Thompson PD, Caspersen CJ, et al (1987). Physical activity and the incidence of coronary heart disease. Ann Rev Public Health 8:253-287.
25. Oldridge NB, Guyatt GH, Fischer ME, et al (1988). Cardiac rehabilitation after myocardial infarction: combined experience of randomized clinical trials. JAMA 260: 945-950.
26. O'Connor GT, Buring JE, Yusaf S, et al (1989). An overview of randomized trials of rehabilitation with exercise after myocardial infarction. Circulation 80:234-244.
27. Sandvick L, Erikssen J, Thoulaw WE, et al (1993). Physical fitness as a predictor of mortality among healthy and middle aged Norwegian men. N Engl J Med 328:5343-5347.

28. Blair SN. Physical activity, fitness, and coronary heart disease. In: Physical activity, fitness and health, Bouchard C, Shephard RJ, and Stephens T (Eds.). Champaign, IL: Human Kinetics, 1994, p.579-590;
29. Hull SS Jr, Vanoli E, Adamson PB, et al (1994). Exercise training confers anticipatory protection from sudden death during myocardial ischemia. Circulation 89:548-552.
30. Myers J (2003). Exercise and cardiovascular health. Circulation 107,e2-e5.
31. Myers J, Prakash M, Froelicher V, et al (2002). Exercise capacity and mortality among men referred for exercise testing. New Engl J Med 346:793-801.
32. Gomez-Alamillo C, Juncos LA, Cases A, et al (2003). Interactions between vasoconstrictors and vasodilators in regulating hemodynamics of distinct vascular beds. Hypertension 42:831-836.
33. Woodman CR, Muller JM, Laughlin MH, et al (1997). Induction of nitric oxide synthase mRNA in coronary resistance arteries isolated from exercise-trained pigs. Am J Physiol 273:H2575-2579.
34. Gattullo D, Pagliaro P, Marsh N, et al (1999). New insight into nitric oxide and coronary circulation. Life Sci 65:2167-2174.
35. Green DJ, Maiorana A, O'Driscoll G, et al (2004). Effect of exercise training on endothelium-derived nitric oxide function in humans. J Physiol 561:1-25.
36. Hägg U, Wandt B, Bergström G, et al (2005). Physical exercise capacity is associated with coronary and peripheral vascular function in healthy young adults. Am J Physiol Heart Circ Physiol 289:H1627-H1634.
37. McCartney N (1998). Role of resistance training in heart disease. Med Sci Sports Exerc 30: S396-402.

38. Billman GE (2009). Cardiac autonomic neural remodeling and susceptibility to sudden cardiac death: effect of endurance exercise training. Am J Physiol Heart Circ Physiol 297:H1171-H1193.

39. Giusti B, Marini M, Rossi L, et al (2009). Gene expression profile of rat left ventricles reveals persisting changes following chronic mild exercise protocol: implications for cardioprotection. BMC Genomics 10:342.

40. Marini M, Lapalombella R, Margonato V, et al (2007). Mild exercise training, cardioprotection and stress genes profile. Eur J Appl Physiol 99:503–510.

41. Murry CE, Jennings RB, Reimer KA (1986). Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. Circulation 74:1124–1136.

42. Pagliaro P, Gattullo D, Rastaldo R, et al (2001). Ischemic preconditioning: from the first to the second window of protection. Life Sci 69:1-15.

43. Yellon DM, Downey JM (2003). Preconditioning the myocardium: from cellular physiology to clinical cardiology. Physiol Rev 83:1113-1151.

44. Brown DA, Moore RL (2007). Perspectives in innate and acquired cardioprotection: cardioprotection acquired through exercise. J Appl Physiol 103:1894–1899.

45. Ferdinandy P, Schulz R, Baxter GF (2007). Interaction of cardiovascular risk factors with myocardial ischemia/reperfusion injury, preconditioning, and postconditioning. Pharmacol Rev 59:418-458.

46. Starnes JW, Taylor RP (2007). Exercise-induced cardioprotection: endogenous mechanisms. Med Sci Sports Exerc 39: 1537–1543.

47. Powers SK, Quindry JC, Kavazis AN (2008). Exercise-induced cardioprotection against myocardial ischemia-reperfusion injury. Free Radic Biol Med 44:193–201.

48. Marongiu E, Crisafulli A (2014). Cardioprotection acquired through exercise: the role of ischemic preconditioning. Curr Cardiol Rev 10:336-348.

49. Varga ZV, Giricz Z, Bencsik P, et al (2015). Functional genomics of cardioprotection by ischemic conditioning and the influence of comorbid conditions: implications in target identification. Curr Drug Targets **DOI**: 10.2174/1389450116666150427154203.

50. Penna C, Mancardi D, Rastaldo R, et al (2009). Cardioprotection: a radical view Free radicals in pre and postconditioning. Biochim Biophys Acta 1787:781-793.

51. Pagliaro P, Gattullo D, Penna C (2013). Nitroglycerine and sodium trioxodinitrate: from the discovery to the preconditioning effect. J Cardiovasc Med (Hagerstown) 14:698-704.

52. Penna C, Angotti C, Pagliaro P (2014). Protein S-nitrosylation in preconditioning and postconditioning. Exp Biol Med (Maywood) 239:647-662.

53. Richardson RS, Duteil S. Wary C, et al (2006). Human skeletal muscle intracellular oxygenation: the impact of ambient oxygen availability. J Physiol 571:415-424.

54. Sõti C, Nagy E, Giricz Z, et al (2005). Heat shock proteins as emerging therapeutic targets. Br J Pharmacol 146:769-780.

55. Milano G, Corno AF, Lippa S, et al (2002). Chronic and intermittent hypoxia induce different degrees of myocardial tolerance to hypoxia-induced dysfunction. Exp Biol Med (Maywood) 227:389-397.

56. Pagliaro P, Penna C (2015). Redox signalling and cardioprotection: translatability and mechanism. Br J Pharmacol;172:1974-1995.

57. Hausenloy DJ, Yellon DM (2007). Reperfusion injury salvage kinase signalling: taking a RISK for cardioprotection. Heart Fail Rev 12:217-234.

58. Hausenloy DJ, Wynne AM, Yellon DM (2007). Ischemic preconditioning targets the reperfusion phase. Basic Res Cardiol 102:445-452.

59. Cohen MV, Downey JM (2011). Ischemic postconditioning: from receptor to end effector. Antioxid Redox Signal 14:821–831.

60. Hausenloy DJ, Lecour S, Yellon DM (2011). Reperfusion injury salvage kinase and survivor activating factor enhancement prosurvival signaling pathways in ischemic postconditioning: two sides of the same coin. Antioxid Redox Signal 14:893-907.

61. Tullio F, Angotti C, Perrelli MG, et al (2013). Redox balance and cardioprotection. Basic Res Cardiol 108:392.

62. Zhao ZQ, Corvera JS, Halkos ME, et al (2003). Inhibition of myocardial injury by ischemic postconditioning during reperfusion: comparison with ischemic preconditioning. Am J Physiol Heart Circ Physiol 285:H579-H588.

63. Tsang A, Hausenloy DJ, Mocanu MM, et al (2004). Postconditioning: a form of "modified reperfusion" protects the myocardium by activating the phosphatidylinositol 3-kinase-Akt pathway. Circ Res 95: 230-232.

64. Penna C, Cappello S, Mancardi D, et al (2006). Post-conditioning reduces infarct size in the isolated rat heart: role of coronary flow and pressure and the nitric oxide/cGMP pathway. Basic Res Cardiol 101:168-179.

65. Penna C, Mancardi D, Raimondo S, et al (2008). The paradigm of postconditioning to protect the heart. J Cell Mol Med 12: 435-458.

66. Pagliaro P, Moro F, Tullio F, et al (2011). Cardioprotective pathways during reperfusion: focus on redox signaling and other modalities of cell signaling. Antioxid Redox Signal 14: 833-850.

67. Ludman AJ, Yellon DM, Hausenloy DJ (2010). Cardiac preconditioning for ischaemia: lost in translation. Dis Model Mech 3: 35-38.

68. Xin P, Zhu W, Li J, et al (2010). Combined local ischemic postconditioning and remote perconditioning recapitulate cardioprotective effects of local ischemic preconditioning. Am J Physiol Heart Circ Physiol 298:H1819-H1831.

69. Hausenloy DJ, Yellon DM (2011). The therapeutic potential of ischemic conditioning: an update. Nat Rev Cardiol 8: 619-629.

70. Przyklenk K, Whittaker P (2011). Remote ischemic preconditioning: current knowledge, unresolved questions, and future priorities. J Cardiovasc Pharmacol Ther 16:255-259.

71. Tamareille S, Mateus V, Ghaboura N, et al (2011). RISK and SAFE signaling pathway interactions in remote limb ischemic perconditioning in combination with local ischemic postconditioning. Basic Res Cardiol 106:1329-1339.

72. Bell RM, Yellon DM (2012). Conditioning the whole heart--not just the cardiomyocyte. J Mol Cell Cardiol 53:24-32.

73. Schoemaker RG, van Heijningen CL (2000). Bradykinin mediates cardiac preconditioning at a distance. Am J Physiol Heart Circ Physiol 278:H1571-H1576.

74. Wang YP, Xu H, Mizoguchi K, et al (2001). Intestinal ischemia induces late preconditioning against myocardial infarction: a role for inducible nitric oxide synthase. Cardiovasc Res 49:391-398.

75. Heusch G, Schultz R (2002). Editorial: remote preconditioning. J Moll Cell Cardiol 34:1279-1281.

76. Gross GJ (2005). Remote preconditioning and delayed cardioprotection in skeletal muscle. Am J Physiol Regul Integr Comp Physiol 289:R1562-R1563.

77. Kuzuya T, Hoshida S, Yamashita N et al (1993). Delayed effects of sub-lethal ischemia on the acquisition of tolerance to ischemia. Circ Res 72:1293-1299.

78. Marber MS, Latchman DS, Walker JM, et al (1993). Cardiac stress protein elevation 24 hours after brief ischemia or heat stress is associated with resistance to myocardial infarction. Circulation 88:1264-1272

79. Bolli R (2000). The late phase of preconditioning. Circ Res 87:972-983.

80. Crisafulli A, Melis F, Tocco F, et al (2004). Exercise-induced and nitroglycerin-induced myocardial preconditioning improves hemodynamics in patients with angina. Am J Physiol Heart Circ Physiol 287:H235-H242.

81. Kalakech H, Tamareille S, Pons S, et al (2013). Role of hypoxia inducible factor-1α in remote limb ischemic preconditioning. J Mol Cell Cardiol 65:98-104.

82. Dost T, Cohen MV, Downey JM (2008). Redox signaling triggers protection during the reperfusion rather than the ischemic phase of preconditioning. Basic Res Cardiol 103:378-384.

83. Becker LB (2004). New concepts in reactive oxygen species and cardiovascular reperfusion physiology. Cardiovasc Res 61:461-470.

84. Asimakis GK, Inners-McBride K, Medellin G, et al (1992). Ischemic preconditioning attenuates acidosis and postischemic dysfunction in isolated rat heart. Am J Physiol Heart Circ Physiol 263:H887-H894

85. Vanden Hoek T, Becker LB, Shao ZH, Li CQ et al (2000). Preconditioning in cardiomyocytes protects by attenuating oxidant stress at reperfusion. Circ Res 86:541-548.

86. Liu Y, Yang XM, Iliodromitis EK, et al (2008). Redox signaling at reperfusion is required for protection from ischemic preconditioning but not from a direct PKC activator. Basic Res Cardiol 103:54-59.

87. Inserte J, Ruiz-Meana M, Rodríguez-Sinovas A, et al (2011). Contribution of delayed intracellular pH recovery to ischemic postconditioning protection. Antioxid Redox Signal 14:923-939.

88. Simkhovich BZ, Whittaker P, Przyklenk K, et al (1995). Transient pre-ischemic acidosis protects the isolated rabbit heart subjected to 30 minutes, but not 60 minutes, of global ischemia. Basic Res Cardiol 90:397-403.

89. Penna C, Rastaldo R, Mancardi D, et al (2006). Post-conditioning induced cardioprotection requires signaling through a redox-sensitive mechanism, mitochondrial ATP-sensitive K+ channel and protein kinase C activation. Basic Res Cardiol 101:180-189.

90. Leeuwenburgh C, Ji LL (1995). Glutathione depletion in rested and exercised mice: biochemical consequence and adaptation. Arch Biochem Biophys 316:941-949.

91. Liu J, Yeo HC, Overvik-Douki E, et al (2000). Chronically and acutely exercised rats: biomarkers of oxidative stress and endogenous antioxidants. J Appl Physiol (1985) 89:21-28.

92. Itoh M, Oh-Ishi S, Hatao H, et al (2004). Effects of dietary calcium restriction and acute exercise on the antioxidant enzyme system and oxidative stress in rat diaphragm. Am J Physiol Regul Integr Comp Physiol 287:R33-R38.

93. Boudreau J, Quadrilatero J, Hoffman-Goetz L (2005). Voluntary training in mice and submandibular lymphocyte response to acute exercise. Med Sci Sports Exerc 37:2038-2045.

94. Mercken EM, Hageman GJ, Schols AM, et al (2005). Rehabilitation decreases exercise-induced oxidative stress in chronic obstructive pulmonary disease. Am J Respir Crit Care Med 172:994-1001.

95. Quadrilatero J, Hoffman-Goetz L (2005). Mouse thymocyte apoptosis and cell loss in response to exercise and antioxidant administration. Brain Behav Immun 19:436-444.

96. Sureda A, Tauler P, Aguiló A, et al (2005). Relation between oxidative stress markers and antioxidant endogenous defences during exhaustive exercise. Free Radic Res 39:1317-1324.

97. McAnulty SR, Nieman DC, Fox-Rabinovich M et al (2010). Effect of n-3 fatty acids and antioxidants on oxidative stress after exercise. Med Sci Sports Exerc 42:1704-1711.;

98. Krüger K, Frost S, Most E, et al (2009). Exercise affects tissue lymphocyte apoptosis via redox-sensitive and Fas-dependent signaling pathways. Am J Physiol Regul Integr Comp Physiol 296:R1518-R1527.

99. Prigol M, Luchese C, Nogueira CW (2009). Antioxidant effect of diphenyl diselenide on oxidative stress caused by acute physical exercise in skeletal muscle and lungs of mice. Cell Biochem Funct. 27:216-222.

100. Lappalainen Z, Lappalainen J, Laaksonen DE, et al (2010). Acute exercise and thioredoxin-1 in rat brain, and alpha-lipoic acid and thioredoxin-interacting protein response, in diabetes. Int J Sport Nutr Exerc Metab 20:206-215.
101. Nie J, Close G, George KP et al (2010). Temporal association of elevations in serum cardiac troponin T and myocardial oxidative stress after prolonged exercise in rats. Eur J Appl Physiol 110:1299-1303.
102. Alessio HM, Hagerman AE, Fulkerson BK et al (2000). Generation of reactive oxygen species after exhaustive aerobic and isometric exercise. Med Sci Sports Exerc 32:1576-1581.
103. Miyazaki H, Oh-ishi S, Ookawara T, et al (2001). Strenuous endurance training in humans reduces oxidative stress following exhausting exercise. Eur J Appl Physiol 84:1-6.
104. Watson TA, MacDonald-Wicks LK, et al (2005). Oxidative stress and antioxidants in athletes undertaking regular exercise training. Int J Sport Nutr Exerc Metab 15:131-146.
105. Bloomer RJ, Fry AC, Falvo MJ et al (2007). Protein carbonyls are acutely elevated following single set anaerobic exercise in resistance trained men. J Sci Med Sport 10:411-417.
106. Fisher-Wellman K, Bloomer RJ (2009). Acute exercise and oxidative stress: a 30 year history. Dyn Med 8:1.
107. Copp SW, Ferreira LF, Herspring KF, et al (2009). The effects of antioxidants on microvascular oxygenation and blood flow in skeletal muscle of young rats. Exp Physiol 94:961-971.

108. Ristow M, Zarse K (2010). How increased oxidative stress promotes longevity and metabolic health: The concept of mitochondrial hormesis (mitohormesis). Exp Gerontol 45:410-418.
109. Ristow M, Schmeisser S (2011). Extending life span by increasing oxidative stress. Free Radic Biol Med 51:327-336.
110. Kojda G, Cheng YC, Burchfield J, et al (2001). Dysfunctional regulation of endothelial nitric oxide synthase (eNOS) expression in response to exercise in mice lacking one eNOS gene. Circulation 103:2839-2844.
111. Lauer N, Suvorava T, Rüther U, et al (2005). Critical involvement of hydrogen peroxide in exercise-induced up-regulation of endothelial NO synthase. Cardiovasc Res 65:254-262.
112. Noble EG, Shen GX (2012). Impact of exercise and metabolic disorders on heat shock proteins and vascular inflammation. Autoimmune Dis 2012:836519.
113. Essig DA, Borger DR, Jackson DA (1997). Induction of heme oxygenase-1 (HSP32) mRNA in skeletal muscle following contractions. Am J Physiol 272:C59-C67.
114. Candilio L, Hausenloy DJ, Yellon DM (2011). Remote ischemic conditioning: a clinical trial's update. J Cardiovasc Pharmacol Ther 16:304-312.
115. Candilio L, Malik A, Hausenloy DJ (2013). Protection of organs other than the heart by remote ischemic conditioning. J Cardiovasc Med (Hagerstown) 14:193-205.
116. Vinten-Johansen J, Shi W (2013). The science and clinical translation of remote postconditioning. J Cardiovasc Med (Hagerstown) 14:206-213.

117. Michelsen MM, Støttrup NB, Schmidt MR, et al (2012). Exercise-induced cardioprotection is mediated by a bloodborne, transferable factor. Basic Res Cardiol 107:260.
118. Jean-St-Michel E, Manlhiot C, Li J, et al (2011). Remote preconditioning improves maximal performance in highly trained athletes. Med Sci Sports Exerc 43:1280-1286.
119. Yamashita N, Hoshida S, Otsu K, et al (1999). Exercise provides direct biphasic cardioprotection via manganese Superoxide dismutase activation. J Exp Med 189:1699–1706.
120. Akita Y, Otani H, Matsuhisa S, et al (2007). Exercise-induced activation of cardiac sympathetic nerve triggers cardioprotection via redoxsensitive activation of eNOS and upregulation of iNOS. Am J Physiol Heart Circ Physiol 292:H2051–H2059.
121. Nelson M, Harris M, Boluyt M, et al (2011). Effect of N-2-mercaptopropionyl glycine on exercise-induced cardiac adaptations. Am J Physiol Regul Integr Comp Physiol 300:R993–R1000.
122. Sanchez G, Escobar M, Pedrozo Z, et al (2008). Exercise and tachycardia increase NADPH oxidase and ryanodine receptor-2 activity: possible role in cardioprotection. Cardiovasc Res 77:380–386.
123. Ji LL, Gomez-Cabrera MC, Vina J (2006). Exercise and hormesis: Activation of cellular antioxidant signaling pathway. Ann NY Acad Sci 1067:425–435.
124. Judge S, Jang YM, Smith A, et al (2005). Exercise by lifelong voluntary wheel running reduces subsarcolemmal and interfibrillar mitochondrial hydrogen peroxide production in the heart. Am J Physiol Regul Integr Comp Physiol 289:R1564–R1572.

125. Powers SK, Criswell D, Lawler J, et al (1993). Rigorous exercise training increases superoxide dismutase activity in ventricular myocardium. Am J Physiol Heart Circ Physiol 265:H2094–H2098.
126. Lennon SL, Quindry JC, French JP, et al (2004). Exercise and myocardial tolerance to ischaemia-reperfusion. Acta Physiol Scand 182: 161–169.
127. Chicco AJ, Hydock DS, Schneider CM, et al (2006). Low-intensity exercise training during doxorubicin treatment protects against cardiotoxicity. J Appl Physiol 100: 519–527.
128. Dickson EW, Hogrefe CP, Ludwig PS, et al (2008). Exercise enhances myocardial ischemic tolerance via an opioid receptor-dependent mechanism. Am J Physiol Heart Circ Physiol 294:H402–H408.
129. Brown DA, Lynch JM, Armstrong CJ, et al (2005). Susceptibility of the heart to ischaemiareperfusion injury and exercise-induced cardioprotection are sex-dependent in the rat. J Physiol 564:619–630.
130. Husain K (2003). Interaction of physical training and chronic nitroglycerin treatment on blood pressure, nitric oxide, and oxidants/antioxidants in the rat heart. Pharmacol Res 48:253–261.
131. Chaves EA, Pereira-Junior PP, et al (2006). Nandrolone decanoate impairs exercise-induced cardioprotection: role of antioxidant enzymes. J Steroid Biochem Mol Biol 99:223–230.
132. Kakarla P, Vadluri G, Reddy KS, et al (2005). Vulnerability of the mid aged rat myocardium to the age-induced oxidative stress: influence of exercise training on antioxidant defense system. Free Radic Res 39:1211–1217.

133. Lawler JM, Kwak HB, Kim JH, et al (2009). Exercise training inducibility of MnSOD protein expression and activity is retained while reducing prooxidant signaling in the heart of senescent rats. Am J Physiol Regul Integr Comp Physiol 296:R1496–R1502.
134. Demirel HA, Powers SK, Zergeroglu MA, et al (2001). Short-term exercise improves myocardial tolerance to in vivo ischemia-reperfusion in the rat. J Appl Physiol 91:2205–2212.
135. Taylor RP, Ciccolo JT, Starnes JW (2003). Effect of exercise training on the ability of the rat heart to tolerate hydrogen peroxide. Cardiovasc Res 58: 575–581.
136. French JP, Hamilton KL, Quindry JC, et al (2008). Exercise-induced protection against myocardial apoptosis and necrosis: MnSOD, calcium-handling proteins, and calpain. FASEB J 22:2862–2871.
137. Ramires PR, Ji LL (2001). Glutathione supplementation and training increases myocardial resistance to ischemia-reperfusion in vivo. Am J Physiol Heart Circ Physiol 281: H679–H688.
138. Jakeman P, Maxwell S (1993). Effect of antioxidant vitamin supplementation on muscle function after eccentric exercise. Eur J Appl Physiol Occup Physiol 67:426-430.
139. Shafat A, Butler P, Jensen RL et al (2004). Effects of dietary supplementation with vitamins C and E on muscle function during and after eccentric contractions in humans. Eur J Appl Physiol. 93:196-202.
140. Gomez-Cabrera MC, Domenech E, Romagnoli M, et al (2008). Oral administration of vitamin C decreases muscle mitochondrial biogenesis and hampers training-induced adaptations in endurance performance. Am J Clin Nutr. 87:142-149.

141. Ristow M, Zarse K, Oberbach A, et al (2009). Antioxidants prevent health-promoting effects of physical exercise in humans. Proc Natl Acad Sci U S A. 106:8665-86670.
142. Yfanti C, Akerström T, Nielsen S, et al (2010). Antioxidant supplementation does not alter endurance training adaptation. Med Sci Sports Exerc 42:1388-1395.
143. Kyparos A, Sotiriadou S, Mougios V, et al (2011). Effect of 5-day vitamin E supplementation on muscle injury after downhill running in rats. Eur J Appl Physiol 111:2557-2569.
144. Theodorou AA, Nikolaidis MG, Paschalis V, et al (2011). No effect of antioxidant supplementation on muscle performance and blood redox status adaptations to eccentric training. Am J Clin Nutr 93:1373-1383.
145. Close GL, Ashton T, Cable T, et al (2006). Ascorbic acid supplementation does not attenuate post-exercise muscle soreness following muscle-damaging exercise but may delay the recovery process. Br J Nutr95:976-981.
146. Kinnunen S, Oksala N, Hyyppä S, et al (2009). alpha-Lipoic acid modulates thiol antioxidant defenses and attenuates exercise-induced oxidative stress in standardbred trotters. Free Radic Res 43:697-705.
147. McAnulty SR, McAnulty LS, Nieman DC, et al (2005). Effect of alpha-tocopherol supplementation on plasma homocysteine and oxidative stress in highly trained athletes before and after exhaustive exercise. J Nutr Biochem 16:530-537.
148. Radak Z, Chung HY, Koltai E, et al (2008). Exercise, oxidative stress and hormesis. Ageing Res Rev 7:34-42.
149. Duncker DJ, Bache RJ (2008). Regulation of coronary blood flow during exercise. Physiol Rev 88:1009-1086.

150. Pagliaro P Exercise and Vascular endothelium functions. In New insight into cardiovascular apparatus during exercise. Physiological and physio-pathological aspects; 207 Research SignPost;
151. Nilius B, Droogmans G (2001). Ion channels and their functional role in vascular endothelium. Physiol Rev 81:1415-1459.
152. Balligand JL, Feron O, Dessy C (2009). eNOS activation by physical forces: from short-term regulation of contraction to chronic remodeling of cardiovascular tissues. Physiol Rev 89:481-534.
153. Baumbach GL. (1991). Is pulse pressure a stimulus for altered vascular structure in chronic hypertension? Hypertension 18:728–729.
154. Awolesi MA, Sessa WC, Sumpio BE (1995). Cyclic strain upregulates nitric oxide synthase in cultured bovine aortic endothelial cells. J Clin Invest 96:1449–1454.
155. Pagliaro P, Senzaki H, Paolocci N, et al (1999). Specificity of synergistic coronary flow enhancement by adenosine and pulsatile perfusion in the dog. J Physiol 520:271-280.
156. Gielen S, Schuler G, Adams V (2010). Cardiovascular effects of exercise training: molecular mechanisms. Circulation 122:1221-1238.
157. Hwang MH, Kim S (2014). Type 2 Diabetes: Endothelial dysfunction and Exercise. J Exerc Nutrition Biochem 18:239-47.
158. Kojda G, Kottenberg K (1999). Regulation of basal myocardial function by NO. Cardiovasc Res 41:514-523.

159. Fukai T, Siegfried MR, Ushio-Fukai M, et al (2000). Regulation of the vascular extracellular superoxide dismutase by nitric oxide and exercise training. J Clin Invest 105:1631-1639.
160. Oltman CL, Parker JL, Adams HR, et al (1992). Effects of exercise training on vasomotor reactivity of porcine coronary arteries. Am J Physiol 263:H372-H382.
161. Prior BM, Lloyd PG, Ren J, et al (2003). Arteriogenesis: role of nitric oxide. Endothelium. 10:207-216.
162. Higashi Y, Sasaki S, Kurisu S, et al (1999). Regular aerobic exercise augments endothelium-dependent vascular relaxation in normotensive as well as hypertensive subjects: role of endothelium-derived nitric oxide. Circulation 100:1194-1202.
163. Maiorana A, O'Driscoll G, Taylor R, et al (2003). Exercise and the nitric oxide vasodilator system. Sports Med 33:1013-1035.
164. Penna C, Granata R, Tocchetti CG, et al (2015). Endogenous Cardioprotective Agents: Role in Pre and Postconditioning. Curr Drug Targets DOI: 10.2174/1389450116666150309115536
165. Ntaios G, Gatselis NK, Makaritsis K, et al (2013). Adipokines as mediators of endothelial function and atherosclerosis. Atherosclerosis 227:216-221.
166. Sakurai T, Ogasawara J, Kizaki T, et al (2013). The effects of exercise training on obesity-induced dysregulated expression of adipokines in white adipose tissue. Int J Endocrinol 2013:801743.
167. Golbidi S, Laher I (2014). Exercise induced adipokine changes and the metabolic syndrome. J Diabetes Res 2014:726861.

168. Maguire JJ, Kleinz MJ, Pitkin SL, et al (2009). [Pyr1]apelin-13 identified as the predominant apelin isoform in the human heart: vasoactive mechanisms and inotropic action in disease. Hypertension 54:598-604.
169. Zhen EY, Higgs RE, Gutierrez JA (2013). Pyroglutamyl apelin-13 identified as the major apelin isoform in human plasma. Anal Biochem 442:1-9.
170. Folino A, Montarolo PG, Samaja M, et al (2015). Effects of apelin on the cardiovascular system. Heart Fail Rev DOI: 10.1007/s10741-015-9475-x
171. Cox CM, D'Agostino SL, Miller MK, et al (2006). Apelin, the ligand for the endothelial G-protein-coupled receptor, APJ, is a potent angiogenic factor required for normal vascular development of the frog embryo. Dev Biol 296:177-1.
172. Kleinz MJ, Davenport AP (2004). Immunocytochemical localization of the endogenous vasoactive peptide apelin to human vascular and endocardial endothelial cells. Regul Pept 118:119-125.
173. Ashley EA, Powers J, Chen M, et al (2005). The endogenous peptide apelin potently improves cardiac contractility and reduces cardiac loading in vivo. Cardiovasc Res 65:73-82.
174. Szokodi I, Tavi P, Földes G, et al (2002). Apelin, the novel endogenous ligand of the orphan receptor APJ, regulates cardiac contractility. Circ Res 91:434-440.
175. Dai T, Ramirez-Correa G, Gao WD (2006). Apelin increases contractility in failing cardiac muscle. Eur J Pharmacol 553:222-228.
176. Farkasfalvi K, Stagg MA, Coppen SR, et al (2007). Direct effects of apelin on cardiomyocytes contractility and electrophysiology. Biochem Biophys Res Commun 357:889-895.

177. Rastaldo R, Cappello S, Folino A, et al (2011). Effect of apelin-apelin receptor system in postischaemic myocardial protection: a pharmacological postconditioning tool? Antioxid Redox Signal 14:909-922.
178. Tatemoto K, Takayama K, Zou MX, et al (2001). The novel peptide apelin lowers blood pressure via a nitric oxide-dependent mechanism. Regul Pept 99:87-92.
179. Hashimoto T, Kihara M, Ishida J, et al (2006). Apelin stimulates myosin light chain phosphorylation in vascular smooth muscle cells. Arterioscler Thromb Vasc Biol 26:1267-1272.
180. Fujie S, Sato K, Miyamoto-Mikami E, et al (2014). Reduction of arterial stiffness by exercise training is associated with increasing plasma apelin level in middle-aged and older adults. PLoS One 9:e93545.
181. Jia YX, Lu ZF, Zhang J, et al (2007). Apelin activates L-arginine/nitric oxide synthase/nitric oxide pathway in rat aortas. Peptides 28:2023-2029.
182. Zhong JC, Yu XY, Huang Y, et al (2007). Apelin modulates aortic vascular tone via endothelial nitric oxide synthase phosphorylation pathway in diabetic mice. Cardiovasc Res 74:388-395.
183. Zhang J, Ren CX, Qi YF, et al (2006). Exercise training promotes expression of apelin and APJ of cardiovascular tissues in spontaneously hypertensive rats. Life Sci 79:1153-1159.
184. Kadoglou NP, Fotiadis G, Kapelouzou A, et al (2013). The differential anti-inflammatory effects of exercise modalities and their association with early carotid atherosclerosis progression in patients with type 2 diabetes. Diabet Med 30:e41-50.

185. Krist J, Wieder K, Klöting N, et al (2013). Effects of weight loss and exercise on apelin serum concentrations and adipose tissue expression in human obesity. Obes Facts 6:57-69.
186. Sheibani S, Hanachi P, Refahiat MA (2012). Effect of Aerobic Exercise on Serum Concentration of Apelin, TNFα and Insulin in Obese Women. Iran J Basic Med Sci 15:1196-201.
187. Besse-Patin A, Montastier E, Vinel C, et al (2014). Effect of endurance training on skeletal muscle myokine expression in obese men: identification of apelin as a novel myokine. Int J Obes (Lond) 38:707-713.
188. Rastaldo R, Cappello S, Folino A, et al (2011). Apelin-13 limits infarct size and improves cardiac postischemic mechanical recovery only if given after ischemia. Am J Physiol Heart Circ Physiol 300:H2308-H2315.
189. Simpkin JC, Yellon DM, Davidson SM, et al (2007). Apelin-13 and apelin-36 exhibit direct cardioprotective activity against ischemia-reperfusion injury. Basic Res Cardiol 102:518-528.
190. Zeng XJ, Zhang LK, Wang HX, et al (2009). Apelin protects heart against ischemia/reperfusion injury in rat. Peptides 30:1144-1152.
191. Wang C, Liu N, Luan R, et al (2013). Apelin protects sarcoplasmic reticulum function and cardiac performance in ischaemia-reperfusion by attenuating oxidation of sarcoplasmic reticulum Ca2+-ATPase and ryanodine receptor. Cardiovasc Res 100:114-124.

192. Pisarenko O, Shulzhenko V, Studneva I, et al (2014). Structural apelin analogues: mitochondrial ROS inhibition and cardiometabolic protection in myocardial ischemia-reperfusion injury. Br J Pharmacol Dec 17. DOI: 10.1111/bph.13038.

193. Pisarenko OI, Lankin VZ, Konovalova GG, et al (2014). Apelin-12 and its structural analog enhance antioxidant defense in experimental myocardial ischemia and reperfusion. Mol Cell Biochem 391:241-250.

194. Foussal C, Lairez O, Calise D, et al (2010). Activation of catalase by apelin prevents oxidative stress-linked cardiac hypertrophy. FEBS Lett 584:2363-70.

195. Frier BC, Williams DB, Wright DC (2009). The effects of apelin treatment on skeletal muscle mitochondrial content. Am J Physiol Regul Integr Comp Physiol 297:R1761-R1768.

196. Mancardi D, Penna C, Merlino A, et al (2009). Physiological and pharmacological features of the novel gasotransmitter: hydrogen sulfide. Biochim Biophys Acta 1787:864-72.

197. Lennon SL, Quindry J, Hamilton KL, et al (2004). Loss of exercise-induced cardioprotection after cessation of exercise. J Appl Physiol (1985) 96:1299-1305.

198. Cosby K, Partovi KS, Crawford JH, et al (2003). Nitrite reduction to nitric oxide by deoxyhemoglobin vasodilates the human circulation. Nat Med 9:1498-1505.

199. Foresti R, Bani-Hani MG, Motterlini R (2008). Use of carbon monoxide as a therapeutic agent: promises and challenges. Intensive Care Med 34:649-658.

200. Vandegriff KD, Young MA, Lohman J, et al (2008). CO-MP4, a polyethylene glycol-conjugated haemoglobin derivative and carbon monoxide carrier that reduces myocardial infarct size in rats. Br J Pharmacol 154:1649-1661.

201. Essig DA, Borger DR, Jackson DA (1997). Induction of heme oxygenase-1 (HSP32) mRNA in skeletal muscle following contractions. Am J Physiol 272:C59-C67.

202. Calvert JW, Condit ME, Aragón JP, et al (2011). Exercise protects against myocardial ischemia-reperfusion injury via stimulation of ß(3)-adrenergic receptors and increased nitric oxide signaling: role of nitrite and nitrosothiols. Circ Res 108:1448-1158.

203. Tavernier G, Toumaniantz G, Erfanian M, et al (2003). beta3-Adrenergic stimulation produces a decrease of cardiac contractility ex vivo in mice overexpressing the human beta3-adrenergic receptor. Cardiovasc Res 59:288-296.

204. Banquet S, Delannoy E, Agouni A, et al (2011). Role of G(i/o)-Src kinase-PI3K/Akt pathway and caveolin-1 in ß-adrenoceptor coupling to endothelial NO synthase in mouse pulmonary artery. Cell Signal 23:1136-1143.

205. Bhambhani Y, Singh M (1991). Physiological effects of hydrogen sulfide inhalation during exercise in healthy men. J Appl Physiol (1985). 71:1872-1877.

206. Yong QC, Lee SW, Foo CS, et al (2008). Endogenous hydrogen sulphide mediates the cardioprotection induced by ischemic postconditioning. Am J Physiol Heart Circ Physiol 2008 295:H1330-H1340.

207. Kang B, Hong J, Xiao J, et al (2014). Involvement of miR-1 in the protective effect of hydrogen sulfide against cardiomyocyte apoptosis induced by ischemia/reperfusion. Mol Biol Rep 41:6845-6853.

208. Abou-Hamdan A, Guedouari-Bounihi H, Lenoir V, et al (2015). Oxidation of H2S in Mammalian Cells and Mitochondria. Methods Enzymol 554:201-228.

209. Veeranki S, Tyagi SC (2014). Role of hydrogen sulfide in skeletal muscle biology and metabolism. Nitric Oxide;46:66-71.

210. de Groot PCE, Thijssen DHJ, Sanchez M et al (2010). Ischemic preconditioning improves maximal performance in humans. Eur J Appl Physiol 108:141-146.

211. Crisafulli A, Tangianu F, Tocco F et al (2011). Ischemic preconditioning of the muscle improves maximal exercise performance but not maximal oxygen uptake in humans. J Appl Physiol 111:530-536.

212. Bailey TG, Jones H, Gregson W et al (2012). Effect of ischemic preconditioning on lactate accumulation and running performance. Med Sci Sport Exerc 44:2084-2089

213. Clevidence MW, Mowery RE, Kushnick MR (2012). The effects of ischemic preconditioning on aerobic and anaerobic variables associated with submaximal cycling performance. Eur L Appl Physiol 112:3649-3654.

214. Gibson N, White J, Neish M, et al (2013) Effect of ischemic preconditioning on land-based sprinting in team-sport athletes. Int J Sports Physiol Perform 8:671-676.

215. Tocco F, Marongiu E, Ghiani G, et al (2015). Muscle ischemic preconditioning does not improve performance during self-paced exercise. Int J Sports Med 36:9-15.

216. Hittinger EA, Maher JL, Nash MS, et al (2015). Ischemic preconditioning does not improve peak exercise capacity at sea level or simulated high altitude in trained male cyclists. Appl Physiol Nutr Metab 40:65-71.

217. Paixao RC, da Mota GR, Marocolo M (2014). Acute Effect of Ischemic Preconditioning is Detrimental to Anaerobic Performance in Cyclists. Int J Sports Med 35;912-915.

218. Lalonde F, Curnier DY (2015). Can anaerobic performance be improved by remote ischemic preconditioning? J Strength Cond Res 29:80-85.

219. Barbosa TC, Machado AC, Braz ID et al (2015). Remote ischemic preconditioning delays fatigue development during handgrip exercise. Scand J Med Sci Sports DOI: 10.1111/sms.12229.

220. Pang CY, Yang RZ, Zhong A, et al (1995). Acute ischemic preconditioning protects against skeletal muscle infarction in the pig. Cardiovasc Res 29:782-788.

221. Reid MB (1985). Invited Review: redox modulation of skeletal muscle contraction: what we know and what we don't. J Appl Physiol 90:724-731.

FIGURE LEGENDS

Figure 1.

Ischemic conditioning consists of a series of brief periods of ischemia and reperfusion performed before (preconditioning), during (perconditioning) or after (postconditioning) the infarcting/index ischemia. The conditioning procedure can be performed in a remote organ with respect to the target organ (e.g. the conditioning protocol can be applied to the limbs and the index ischemia to the heart).

Figure 2

The release of several endogenous cardioprotective agents. Cardioprotective agents can be released by several cell types and may act in a paracrine/autocrine fashion to activate membrane receptors and to trigger redox-sensitive intracellular pro-survival pathways. Cardioprotective pathways converge on mitochondria where they prevent mitochondrial permeability transition pore (mPTP) formation.

Adenosine receptors, AR; Bradykinin receptors, BkR; Opioid receptors, OpR, Apelin receptor APJ. For other acronyms see text.